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THE SECRETORY INNERVATION OF
THE DOG'S PAROTID GLAND

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by

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4. Holmberg, J. (1972). Secretion from the dog's parotid at different time intervals after denervation. Acta physiol. scand. 85, 305-311.
5. Ekström, J. and Holmberg, J. (1972). Choline acetyltransferase in the normal and parasympathetically denervated parotid gland of the dog. Acta physiol. scand. (in the Press).
6. Ekström, J. and Holmberg, J. (1972). Effect of decentralization on the choline acetyltransferase of the canine parotid gland. J. Physiol. 222, 93P.
7. Holmberg, J. (1972). Release of acetylcholine in the parotid gland of the dog during stimulation of postganglionic nerves. Acta physiol. scand. 86, 115-119.



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INTRODUCTION

Salivary glands have been used in a great number of investigations devoted to the function of the autonomic nervous system. There are no doubt several reasons for this. The glands are paired and well defined organs; as far as known they are normally activated exclusively by nerves, and it is easy to record the glandular activity as flow of saliva. The submandibular gland has been much more studied than the parotid gland in spite of the fact that it has a definite disadvantage in experiments on the secretory innervation: the synapses between pre- and postganglionic parasympathetic neurones are situated very close to the gland or within the glandular tissue. In the case of the parotid gland, on the other hand, the postganglionic neurones seem to be more accessible for stimulation and section. The difficulty with this gland, however, is that the course of the secretory nerves is not well known, although it has been a subject of interest to many physiologists for about a century.

Ludwig discovered the secretory nerve to the dog's submandibular gland as early as 1851, but the dependence of the parotid secretion upon such nerves was not clear to the early physiologists. In the words of Claude Bernard (1879) "on la considérait comme absolument passive dans l'acte de l'insalivation; tous les physiologistes la regardaient comme une sorte d'éponge qui se trouvait comprimée par les mouvements de mastication de l'animal". "Après une longue et laborieuse série d'expériences" he could demonstrate, however, that parotid secretion, evoked by pouring vinegar on the tongues of conscious dogs was abolished when the auriculo-temporal nerve was divided, whereas electrical stimulation of the peripheral

end of the cut nerve evoked an abundant secretion from the gland. When he forced a sharp instrument through the tympanic cavity into the petrosal bone, thereby dividing the facial nerve in the bony canal, reflexly induced parotid secretion was, likewise, found to be annulled. These results were known by Schiff already in 1858, but were not published until 1879. Claude Bernard formed the conception, mainly based on these experiments, that the parotid secretory fibres originate in the facial nerve and then travel via the smaller superficial petrosal nerve, the otic ganglion and the auriculo-temporal nerve to the gland. In his opinion the cervical sympathetic trunk does not contain secretory fibres for the dog's parotid, because he could not provoke any parotid secretion in this species by stimulating it electrically, an observation which was also made by Navrocki (1868) and Heidenhain (1878).

Claude Bernard's findings on dogs were confirmed in this or some other species both regarding the origin of the secretory fibres in the facial nerve (Schiff, 1858; Czermak, 1860) and their further course in the small superficial petrosal (Schiff, 1858) and auriculo-temporal nerves (Schiff, 1858; Navrocki, 1868). Conflicting results, however, were obtained by Eckhard (1863). He found the parotid secretory response to reflex stimulation to be unaffected by intracranial division of the facial nerve, and his pupil Loeb (1869) demonstrated that it was abolished after intracranial section of the glossopharyngeal nerve. When Heidenhain (1878) obtained a copious secretion consequent on electrical stimulation of the tympanic branch of this nerve, the original conception of Claude Bernard was modified. The secretory fibres to the dog's parotid seemed to originate not in the facial, but in the glossopharyngeal nerve, to branch off within the jugular foramen as the tympanic nerve (of Jacobson) and then to travel

as the smaller superficial petrosal nerve via the otic ganglion and the auriculo-temporal nerve to the gland.

This classical conception, founded on observations mainly in dogs and later extended also to other species, is not easy to reconcile with results from some more recent experiments, and in 1950 Babkin wrote in his monograph *Secretory Mechanism of the Digestive Glands*: "The whole problem of the innervation of the salivary glands requires re-investigation". He describes experiments in dogs, where 20-25 percent of the normal parotid secretory response to a meal was obtained after extirpation of the tympanic nerve (Andrejev & Podkopayev, 1928) and some 20 percent after division of the auriculo-temporal nerve (Baxter). In some way the time factor seemed important, because the secretory response in both cases increased in the course of time. Babkin (1950) considers the possibility of "reserve paths" which after section of the chief secretory nerve gradually take over the function of this nerve. It has recently been confirmed that the dog's parotid receives secretory fibres which travel outside the auriculo-temporal nerve. Thus one week after divisions of the latter nerve on the medial side of the mandible as described by Burgen (1964) a considerable parotid secretion could be obtained in response to lemon juice, poured on the tongue of dogs in light thiopentone anaesthesia, and numerous cholinesterase positive nerves were still found in the "denervated" glands (Emmelin, Garrett & Holmberg, 1968).

The main purpose of the present investigation was to find the hitherto unknown extraglandular course of the secretory fibres, which, according to the findings just discussed, seem to reach the parotid gland of the dog via other routes than the auriculo-temporal nerve. First various cranial nerves, suspected to

carry parotid secretory fibres, were electrically stimulated in acute experiments as described in Chapter 1. A large amount of such fibres were thus traced on the internal maxillary artery and a small amount in the facial nerve. Reflexly induced secretion was then recorded before and after section of the nerves found, and the auriculo-temporal nerve, in order to find out whether the gland can be activated via other, still unknown pathways. These experiments are summarized in Chapter 2. Chapter 3 deals with the synthesis and release of acetylcholine in the gland.

CHAPTER 1

ELECTRICAL STIMULATION EXPERIMENTS

METHODOLOGICAL CONSIDERATIONS

To facilitate quantitative comparisons between the animals, the secretory responses to electrical stimulation of the different nerves in a certain dog were expressed as percent of the maximal secretory rate that could be induced by reflex activation in the same animal. The classical Pavlov method of recording reflex secretion in conscious chronic fistula dogs was considered unsuitable because of the difficulties involved in quantitative collection of the saliva and the hazards of stricture formation and ascending infection in these preparations. A maximal activation of the gland was desirable, so Gregersen's (1931) method of thermal activation was also discarded. Instead, the dogs were anaesthetized with an i.v. injection of a short-acting barbiturate (sodium thiopentone), the parotid ducts were cannulated from the mouth, and the cannulae connected to a recording system. When the blinking reflex reappeared citric acid was poured repeatedly on the tongues of the animals for periods of 1-2 min until no further increase in salivary flow rate could be recorded. The highest secretory rate obtained during 30 s was chosen as the maximal reflexly induced secretory rate (1).

The electrical stimulation experiments were carried out in chloralose-urethane anaesthesia. The nerves, suspected or known to contain parotid secretory fibres were divided and the peripheral stumps were stimulated with bipolar electrodes. An exception was the fragile tympanic nerve, the central connections of which were not divided in most experiments

and which was stimulated with a small open bipolar electrode. The nerves were stimulated using different frequencies and supramaximal intensities for periods of 1 min, or more if the secretion obtained was scanty. The fastest secretory rate recorded during 30 s was expressed as percent of the maximal reflexly induced secretory rate of the individual gland.

No significant irradiation occurred from the stimulating electrodes to surrounding secretory nerves; there was no parotid secretion when the nerves in the electrodes were stimulated after firm ligation or division of the nerves between the electrode and the gland (1; 2).

RESULTS

Nerves travelling with the internal maxillary artery

The auriculo-temporal nerve was exposed from the ventral side of the neck as described by Burgen (1964) and the exposition was widened to include the trunk of the mandibular nerve proximal to the origin of the auriculo-temporal nerve (Fig. 1 and paper nr. 1, Fig. 4). It was found that parotid secretion could be induced by stimulation of fibrous strands which, running in the same direction as the auriculo-temporal nerve, connect the mandibular trunk with the internal maxillary artery. Some of these strands can no doubt be considered as part of the root of the auriculo-temporal nerve, which is commonly represented in text-books of (human) anatomy as embracing the artery. Secretion could be induced, however, by stimulating such strands some 5 mm both medial and lateral to the origin of the auriculo-temporal nerve. It was then suspected that some of these strands contained secretory fibres which did not join the auriculo-temporal nerve. It was not possible to divide this nerve at its origin without

destroying the connecting strands, but when it had been divided as close to its origin as possible, at the mandibular joint, parotid secretion could still be induced by electrical stimulation of the mandibular nerve (1). Evidently, this nerve contained secretory fibres, which were not present in the auriculo-temporal nerve at the mandibular joint. These fibres apparently joined the internal maxillary artery, and stimulation of this structure caused parotid secretion. In fact, stimulation of the artery and the auriculo-temporal nerve at the mandibular joint provoked secretory responses of equal magnitude, suggesting that the amount of secretory fibres travelling with the two structures was similar at that level. Stimulation of the mandibular nerve when the auriculo-temporal nerve had been divided at the joint evoked less ($1/3 - 1/10$) secretion than did excitation of the auriculo-temporal nerve. This can be explained by assuming that only part of the secretory fibres present on the artery at the level of the mandibular joint is derived directly from the mandibular nerve, whereas part of them is given off from the auriculo-temporal nerve at the joint.

Already Claude Bernard (1879) described secretory fibres on the internal maxillary artery: "Il est en rapport intime avec l'artère maxillaire interne, dont il suit exactement le trajet, mais en sens inverse du cours du sang". In his opinion, these fibres detach themselves from the auriculo-temporal nerve. As discussed above, the present investigation shows that many of them do, but that some of them apparently pass directly from the mandibular nerve to the artery. It seems that Claude Bernard's finding of secretory fibres on the artery has not attracted the attention it deserves; in large reviews on the control of salivary secretion such as Babkin's in "Secretory Mechanism of the Digestive Glands" (1950) and

Emmelin's in "Handbook of Physiology" (1967) considerable space is devoted to unknown nerves to the parotid gland, but secretory fibres on the artery are not discussed in this connexion. According to Claude Bernard (1879) reflexly induced parotid secretion was abolished after division of the auriculo-temporal nerve. He did not state where he severed the nerve, but according to the present findings this is an essential point; section at the origin will include many of the fibres on the artery, whereas division at the mandibular joint, or more peripherally, will leave most of them intact.

The secretory fibres both on the artery and in the auriculo-temporal nerve were found to be postganglionic; the secretory responses to stimulation of them were unaffected by hexamethonium. The secretion was totally abolished by a small dose of atropine (0.1-0.2 mg/kg), indicating that all the nerves studied were cholinergic (1).

It appeared that these secretory nerves partially converged on the same glandular cells. By means of simultaneous electrical stimulation of the auriculo-temporal nerve and of the fibres on the internal maxillary artery with a frequency of 20-30/s it was often possible to obtain the same rate of parotid secretion as could maximally be induced by reflex stimulation. The summated secretory rates provoked by excitation of each of the two sets of nerves separately with this frequency, however, exceeded the maximal rate attained by reflex stimulation. When, on the other hand, only 2 shocks/s were applied to the nerves the reverse occurred, i.e. the sums of the separately provoked responses were smaller than the responses obtained on simultaneous excitation (1).

The functional organization of the secretory innervation within salivary glands has been more extensively studied in the

cat's submandibular gland. Thus, in this gland, Ekström and Emmelin (1971) demonstrated a marked convergence of the post-ganglionic nerves. Lundberg (1955) on the basis of electrophysiological evidence suggested that each cell in the cat's submandibular receives 5-10 nerve axons, whereas Creed and Wilson (1969) using electronmicroscopical technique calculated that in the same gland 2-5 junctions are associated with each cell. The neuro-effector junctions in this gland are considered to be sites, where the axons travel free from their Schwann cell investment near (Garrett, 1966) or between (Creed & Wilson, 1969) the acinar cells. The electronmicroscopical appearance of the neuro-effector sites in the dog's parotid is very similar (Garrett, personal communication).

Although the axons from the auriculo-temporal nerve thus partly make functional contact with the same secretory cells as those on the artery, it is usually not possible to attain the maximal reflexly induced secretory rate by stimulating only the auriculo-temporal nerve (Emmelin & Holmberg, 1967). Such high a rate of secretion could, as mentioned above, often be attained by the simultaneous excitation of the two sets of nerves, but by no means always; it was, in fact, obtained only in half the number of dogs studied (1). Probably this was mainly due to technical difficulties; there is an abundance of veins in the region studied and the nerves had to be dissected and stimulated in a deep and narrow cavity, slowly filling with blood. Some fibres might have escaped notice or been destroyed during the dissection. The fairly extensive preparations might also have caused impairment in the circulatory state of the animal or locally in the gland and it is well known that high rates of salivary secretion can only be induced if the gland is well supplied with blood. It might also be, however, that there were glandular cells which were

not maximally activated by the two nerves discussed, but wholly or partly via some other nerve.

The facial nerve

Electrical stimulation of the facial nerve elicited parotid secretion in most of the dogs investigated (5). This nerve thus contains secretory fibres, but much fewer than the two other nerves described; on excitation of either the auriculo-temporal nerve or the nerves on the artery the gland produced about 20-30 drops/min, whereas, on the average, only 1 drop in 5 min was obtained when the facial nerve was stimulated at the same frequency. It should be pointed out that the minute secretory responses to stimulation of the facial nerve persisted after gallamine and were thus not due to contraction of the facial muscles partly covering the gland. They were, however, abolished by atropine, indicating that the secretory nerves were cholinergic. Experiments with ganglion blocking drugs were difficult to interpret, probably mainly because of the small size of the responses. In most of the glands the responses were somewhat reduced after injection of hexamethonium (5). This may imply that there were some synapses distal to the stimulating electrode, extra- or intraglandularly. The reduction of the secretion might, however, also be a consequence of impaired glandular circulation caused by the lowering of the blood pressure induced by the ganglion blocker. In histological sections of the gland no ganglion cells were found (Garrett & Holmberg, 1972).

Other postganglionic nerves

Despite extensive search no parotid secretory nerves were found except those described (and the tympanic nerve). Stimulation of the chorda tympani in the tympanic cavity or just outside

the Glaserian fissure caused no secretion, nor did excitation of the peripheral stump of the glossopharyngeal nerve divided near the jugular foramen (1). The main parotid secretory nerve of ox and sheep travels along the parotid duct (Moussu, 1890). It seemed to have no counterpart in the dog, because stimulation along the duct caused no secretion (Holmberg, unpublished observation). Occasionally, in superficial anaesthesia, some secretion was provoked by stimulation of the vagosympathetic trunk in the neck. It was always associated with arousal and signs of nausea (1). It has been shown by Emmelin, Ohlin and Thulin (1969) that such secretion is caused by afferent stimulation of the vagus and that the efferent pathway is cholinergic. Stimulation of the cervical sympathetic separated from the vagus just proximal to the superior cervical ganglion or postganglionically on the external carotid artery did not cause parotid secretion. Vasoconstrictor and myoepithelial cell responses in the gland served as a control of the efficiency of the stimulation (Holmberg, unpublished observation). Most investigators (Bernard, 1879; Heidenhain, 1878; Babkin, 1950; Emmelin, Ohlin & Thulin, 1969) agree that stimulation of the cervical sympathetic does not evoke parotid secretion in the dog.

The preganglionic nerve

Parotid secretion was evoked when the tympanic nerve was stimulated. It was abolished by hexamethonium, indicating that the nerve was preganglionic (2).

The parotid secretion induced by stimulation of the nerve was at least as large as that provoked by simultaneous excitation of the auriculo-temporal nerve and the nerves on the internal maxillary artery and it was usually possible to attain the maximal reflexly induced secretory rate of the gland (2). Thus

the overwhelming part of the parotid secretory cells could be activated via the tympanic nerve. The secretory pathway from this nerve turned out to continue mainly in the postganglionic nerves described; when the auriculo-temporal nerve was divided at the level of the mandibular joint, the response to stimulation of the tympanic nerve was reduced. The remaining response, mediated via other nerves, was of a magnitude of about half the initial one although it varied considerably. It was reduced to around 1 percent of the initial response when also the internal maxillary artery was divided at the same level. (As can be deduced from Fig. 1 this manoeuvre does not interrupt the blood supply to the gland). This small remaining secretion could be further diminished by section of the facial nerve just outside the stylomastoid foramen (2). The conclusions drawn from the experiments with direct stimulation of the postganglionic nerves could thus be confirmed, viz. the amount of parotid secretory fibres in the auriculo-temporal nerve and on the internal maxillary artery are about equal at the level of the mandibular joint, and these structures carry a much larger number of secretory fibres than the facial nerve. When all these nerves had been divided, stimulation of the tympanic nerve still caused a minute parotid secretion. This fraction was abolished by atropine and was thus mediated by cholinergic fibres. The peripheral course of these fibres has not been traced.

CHAPTER 2

DENERVATION EXPERIMENTS

Stimulation experiments described in the previous Chapter show that parotid secretion can be elicited in the dog by electrical excitation not only of the tympanic and auriculotemporal nerves but also of fibres on the internal maxillary artery and in the facial nerve. They also hint that there may be other secretory pathways. In order to throw some further light on the secretory innervation, and particularly to find out whether the nerves so far described form the main efferent link of the secretory reflex arc, denervation experiments were carried out.

METHODOLOGICAL CONSIDERATIONS

Parotid secretion was elicited reflexly as in the previously described experiments, and the effects of section of nerves were examined. This was not all done in an acute experiment, since cutting the nerves often involved fairly extensive prior dissection; the surgical trauma might in itself reduce the secretory responses, as discussed above. Instead, the effect on the secretion was studied 2-6 days after division of the nerves, when the animals had fully recovered from the operation. This method of ascertaining whether there are parotid secretory nerves beside those severed is direct and simple, but it has its complications and limitations. There is, of course, a limit to the sensitivity of the method; if the secretory nerves left are too few to be able to exert an effect by which the secretory threshold is surpassed, even though they are maximally activated, then they will escape detection. Furthermore, in denervated salivary glands some phenomena

occur, which might cause secretion even if all the secretory nerves have been divided, or act to increase the secretory responses to activation of remaining nerves, thus complicating the quantitative estimation of the normal contribution to secretion of these nerves. These phenomena, degeneration secretion, supersensitivity of denervation and early nerve regeneration therefore had to be investigated. The degeneration secretion was recorded in dogs in chloralose-urethane anaesthesia 1-3 days after division of the postganglionic nerves. To study the other two phenomena, experiments were made before, and repeatedly after cutting the nerves according to the following procedure. First maximal reflexly induced secretory rate was induced as described above. Then the anaesthesia was deepened with an additional dose of the barbiturate; hexamethonium, 10 mg/kg, was given intravenously, and the sensitivity of the gland to methacholine was estimated by injection of standardized doses of the drug. Finally, eserine was injected in retrograde direction into the parotid duct. In innervated glands this causes secretion by preserving the acetylcholine, "leaking" from nerves even in the absence of nerve impulses (Emmelin & Perec, 1968).

RESULTS

Early effects of denervation

Reflexly induced secretion. When only the auriculo-temporal nerve had been divided at the mandibular joint 2 days prior to the experiment, the maximal reflexly induced secretory rate was about half the normal one (4). In 9 dogs out of 16 investigated, reflexly induced secretion was abolished 3-5 days after section of both the auriculo-temporal nerve and the fibres on the artery (1). In the others, the maximal se-

cretory response of the gland, in percent of the preoperative value was: 4, 6, 12, 17, 18, 20, 32. As would be expected from the electrical stimulation experiments, section of the facial nerve in addition to the other two sets of nerves further reduced reflexly induced secretion (5); in four animals out of six it was annulled; in the other two dogs 5 and 6 percent, respectively, of the normal secretion remained.

The most important conclusion in this context drawn from these experiments is that the overwhelming part of the parotid secretory fibres are present on the internal maxillary artery and in the auriculo-temporal and facial nerves, and a very small part of the secretory fibres might travel by some other route. Furthermore, these findings support the concept based on the electrical stimulation experiments regarding the relative number of secretory nerves in the three main sets of nerve; the auriculo-temporal secretory pathway and the one on the artery contain about equal amounts of secretory fibres and far more fibres than the facial nerve. The very small secretory responses which could be induced in some of the dogs where these three sets of nerve had been cut might of course have been mediated via fibres, unintentionally left on the artery, which had to be handled with considerable care when the nerves were stripped off. This is not likely to be the whole explanation, however, because, as described in Chapter 1, small secretory responses remained to electrical stimulation of the tympanic nerve in acute experiments when the artery was not stripped but divided together with the auriculo-temporal and facial nerves. This finding indicates the existence of a minute secretory pathway in the tympanic nerve which runs outside the auriculo-temporal and facial nerves and those on the artery. The arrangement of the secretory fibres of the gland is, indeed, complex. There seem to be

still other unknown secretory fibres, which contrary to those so far discussed, do not travel in the tympanic nerve, because when the latter one had been cut (2), reflexly induced secretion was abolished only in five glands out of ten, whereas, in the others, some secretion was still present (3, 3, 5, 6 and 12 percent of the normal reflex response). Andreyev and Podkopayev (1928) reported that after the same kind of operation, 20-25 percent of reflexly induced parotid secretion remained in chronic fistula dogs, whereas Loeb (1869) noted complete abolishment of the secretion after acute division of the nerve. The varying results might be explained by the different methods used. Individual variation and the small number of experiments also make comparison difficult. The present denervation and electrical stimulation experiments indicate that the tympanic nerve is by far the most important preganglionic secretory nerve, but that there might be other pathways too, at least in some dogs.

The stimulation experiments showed that secretion caused by activation of the auriculo-temporal nerve, the nerves on the internal maxillary artery and the facial nerve was abolished by small doses of atropine, and these secretory nerves were hence assumed to be cholinergic. It could be suspected that the small secretory response that could sometimes still be elicited reflexly after section of these nerves was mediated by adrenergic nerves. This did, however, not seem very likely in view of the fact that sympathetic nerve stimulation did not cause any secretion, and the possibility was excluded by the finding that secretion induced by citric acid on the tongue was totally abolished by atropine, both in normal and partly denervated glands (1; 2).

Degeneration secretion. In salivary glands, section of the

postganglionic glandular nerves is followed after 1 or 2 days by secretory activity, which may last for a day or more. This "degeneration secretion" is due mainly to an increased transmitter leakage from the degenerating nerves (see Emmelin & Trendelenburg, 1972). Since this could be thought to complicate matters when secretion was studied a few days after nerve section, a series of experiments was carried out (3) to learn whether the phenomenon occurs in parotid glands of dogs, where it had so far not been studied. Some degeneration secretion was obtained, starting about 2 days after denervation. The flow was, however, much slower and of shorter duration than in, for instance, the submandibular gland of the dog (Coats & Emmelin, 1962). It may be that a small secretory response obtained reflexly during the period of degeneration secretion is increased and the number of secretory fibres remaining after attempted denervation thus somewhat overestimated, but on the whole the phenomenon did not seem to interfere seriously with the estimations of the reflexly elicited responses, as will be discussed in the next section of this Chapter.

Late effects of denervation

Supersensitivity. The sensitivity of the parotid gland of the dog was found to rise to a high level after section of the auriculo-temporal nerve or this nerve and the fibres on the internal maxillary artery. Greatly increased responses to methacholine were obtained already 2-4 days after nerve section (4). It seems reasonable to assume, as discussed in paper (4), that at this stage the enhanced effects were related to the increased leakage of transmitter during the period of degeneration secretion rather than to a supersensitivity developed in the glandular cells. It is therefore difficult

to state when such a supersensitivity first appeared and when it reached a maximum. The experiments showed, however, that over a period of five weeks following the denervation there was no decline in the level of sensitivity. Hence, these experiments did not give any indication of a functional re-innervation occurring during this time.

Effects of eserine. When eserine was applied locally in the gland under standardized conditions the following observations were made (4). After a rise, obtained 2 days after denervation, the responses decreased to a low level, reached after one week. The transient rise above the control value can be connected with the temporarily increased transmitter leakage while divided fibres were degenerating. The responses then obtained were due to acetylcholine leaking from remaining postganglionic nerves. After section of the auriculo-temporal nerve the effects of eserine were greatly reduced and in glands where both this nerve and the fibres on the artery had been severed there was a still larger reduction showing that section of each set of nerves deprived the gland of a great number of cholinergic nerves. Even after the more extensive denervation eserine had often some small secretory effect, suggesting that there were still some cholinergic fibres left in the gland. These observations are thus in general agreement with those made when secretion was elicited by efferent nerve stimulation or reflexly. During the observation period of 5 weeks there was no marked increase of the responses to eserine; this suggests that no significant functional re-innervation had taken place.

Reflexly induced secretion. The rate of salivary flow elicited reflexly was found to remain fairly unchanged when studied at intervals over a period of 5 weeks following section of the

auriculo-temporal nerve or this nerve and the fibres on the internal maxillary artery (4). This may seem surprising considering the facts that the operation was followed by a temporary increase in the transmitter leakage which was succeeded by supersensitivity in the glandular cells. The explanation may be that the cells, first exposed to increased amounts of transmitter and then the sites of supersensitivity were not activated, or not sufficiently activated, by the remaining secretory nerves when these were excited reflexly. Alternatively, it is possible that the reflexly induced responses were in fact somewhat exaggerated during the whole observation period, first by the increased transmitter leakage and then by the supersensitivity.

It seems, at any rate, safe to conclude that during the first 5 weeks there is no pronounced increase in the responses that can be elicited reflexly, and the experiments on the sensitivity to methacholine and the effect of eserine support the view that there is no marked functional reinnervation. There are some observations suggesting that collateral regeneration can occur in salivary glands. Nordenfelt (1965a) observed an increase in the choline acetyltransferase activity in parotid glands of cats following sympathectomy, which he tentatively explained as due to sprouting from cholinergic axons, induced from the degenerating sympathetic neurones. Similar observations have been made in other salivary glands (Nordenfelt, 1964a; Ekström, 1972); in the parotid gland of the dog an increase in the enzyme content occurred within 5 weeks after sympathetic ganglionectomy (Ekström, 1972). It may be that in the present experiments sprouting actually occurred from remaining postganglionic cholinergic neurones but that functional contact was not to any great extent established during the period of study. Experiments on denervation super-

sensitivity and secretory responses to eserine made on submandibular glands of cats suggest that collateral regeneration can take place in this gland (Emmelin & Perec, 1968) but the time scales of those experiments and the present ones are not comparable, since in the submandibular glands preganglionic contact had to be restored before sprouting seemed to occur.

In the present study there is thus no indication of marked reinnervation or mobilization of "reserve paths" (Babkin, 1950) within the period of observation. The results differ in this respect from those of Baxter. He found that the secretory response to food from parotid fistulae in dogs was "very scanty" during the first few days after transection of the auriculo-temporal nerve but that it gradually increased to about one fifth of that of the normal gland on the 10th day. It should be pointed out that food as a stimulus to secretion provokes a less intense activation of the secretory cells than do rejectable substances (Emmelin & Holmberg, 1967) such as citric acid. This might explain some of the differences in the results. It is not stated where Baxter divided the auriculo-temporal nerve, but if, in fact, he cut it on the lateral side of the mandible where it is most easily exposed, then postoperative, periglandular oedema might well have been responsible for the smallness of the secretory responses which he recorded during the first 10 postoperative days.

CHAPTER 3

ACETYLCHOLINE SYNTHESIS AND RELEASE IN THE GLAND

Experiments described in the previous Chapters indicate that flow of saliva from the dog's parotid gland, elicited reflexly or by efferent nerve stimulation, is evoked exclusively by way of cholinergic nerves. It is of obvious interest, then, to study the acetylcholine synthesizing capacity of the gland before and after section of nerves, and to gain some information about the amounts of acetylcholine released in the gland on stimulation of the nerves. It is, of course, important to keep in mind that there may be other cholinergic neurones in the gland than those representing the secretory pathway, for instance vasodilator nerves and axons supplying myoepithelial cells. Conclusions from comparisons as to quantities of acetylcholine synthesized or released under various conditions should therefore be drawn with caution. It is possible, for example, that the proportion between cholinergic axons of different function is different in the various glandular nerves studied and that the capacity to synthesize or release acetylcholine varies from one type of axon to another.

METHODOLOGICAL CONSIDERATIONS

The method for choline acetyltransferase estimation was that of Catherine Hebb, described in detail by Nordenfelt (1965b). Bioassay of the acetylcholine synthesized was carried out on the frog rectus muscle (Feldberg, 1950).

To study acetylcholine release, a preparation was worked out in which the gland was perfused from the arterial side with heparinized, eserinizd dog plasma. Estimation of acetylcho-

line in the perfusate recovered from the venous side was carried out on the blood pressure of eviscerated cats as described by MacIntosh and Perry (1950).

RESULTS

Synthesis of acetylcholine

Like other salivary glands (Nordenfelt, 1965b) the dog's parotid gland was found to be able to synthesize acetylcholine (5). The capacity was fairly low, however. It was, for instance, considerable lower than that of the submandibular gland of the dog (Nordenfelt, 1964a). This difference is probably partly due to the presence of cholinergic ganglion cells in the submandibular but not in the parotid gland.

Section of each of the nerves known to carry secretory fibres to the gland was found to reduce the acetylcholine synthesizing capacity (5). After the division of the auriculo-temporal nerve the activity of the enzyme was decreased to about 30 percent of normal. Division of both this nerve and the fibres on the internal maxillary artery lowered the value to about 10 percent, and when the facial nerve and the two other sets of nerve were cut, the activity was reduced down to the limit of detection. Obviously all these nerves contained cholinergic fibres. The amount of cholinergic fibres remaining when these three sets of nerve had degenerated was apparently very small, corresponding to the finding that in such glands very little secretion, or none at all, could be elicited reflexly.

In parotid glands of cats Nordenfelt (1964b) made the interesting discovery that the acetylcholine synthesizing capacity was dependent on the connection with the central nervous system. Section of the preganglionic parasympathetic neurone

reduced the choline acetyltransferase activity, and it was suggested that impulse traffic in the pathway influenced the enzyme. The existence of such an influence on the transmitter and its synthesis has also been discussed in the case of adrenergic nerves (see Pletscher, 1972). The preparation used by Nordenfelt, the parotid gland of the cat, has the disadvantage that preganglionic parasympathetic denervation, by section of the tympanic nerve, to a varying degree also entails a postganglionic sympathetic denervation. This is a complicating factor, for sympathectomy increases in itself the choline acetyltransferase activity of this gland, as shown by Nordenfelt (1965a) and discussed in Chapter 2. In dogs the tympanic nerve does not contain any sympathetic fibres to the parotid gland (Garrett & Holmberg, 1972). Using this preparation the findings made by Nordenfelt (1964b) regarding the influence of the preganglionic neurone on the synthesizing capacity could be confirmed (6).

Acetylcholine release

Electrical stimulation of the nerves of the gland was found to cause a liberation of acetylcholine (7). This finding is of some general interest for the reason that there are fairly few preparations in which it is possible to study transmitter release on electrical stimulation of postganglionic parasympathetic nerves. Mostly preganglionic parasympathetic nerves have to be stimulated, as for instance in the fundamental experiment on the heart by Loewi (1921), and the preganglionic fibres are assumed to be cholinergic also and hence to contribute to the amounts of acetylcholine released on nerve stimulation. Acetylcholine from postganglionic nerves has been collected separately in the eye (Engelhart, 1931) and in the rat's urinary bladder (Carpenter & Rand, 1965; Chesher,

1967); in the submandibular gland the two fractions of acetylcholine have been separated by pharmacological means (Emmelin & Muren, 1950).

When the auriculo-temporal nerve and the nerves on the internal maxillary artery were stimulated together, the quantities of acetylcholine set free were twice as large as those obtained when only the auriculo-temporal nerve was excited (7). It seems reasonable to connect this with the previous findings indicating that similar amounts of secretory axons are present in the two sets of nerves.

The amounts of transmitter released when both nerves were stimulated together were considerably smaller than those obtained from the submandibular gland on stimulation of the chorda tympani (7). This seemed to be true even if only acetylcholine from the postganglionic endings in the submandibular gland was taken into account. This is in agreement with the fact that this gland has a smaller acetylcholine synthesizing capacity than the parotid gland, as discussed above.

When the quantities of acetylcholine obtained on nerve stimulation were compared with those which the gland was able to synthesize it was evident that there is a high safety factor in the transmission process, as previously shown in the cat's submandibular gland (Nordenfelt, 1967). On nerve stimulation about 3 μg acetylcholine could be calculated as released per hour; very likely this figure does not represent the maximum, considering the unphysiological conditions of the perfusion experiments, but for comparison it may be mentioned that in the in vitro experiments the parotid gland was found to be able to synthesize as much as about 150 μg acetylcholine per hour (5).

COMMENTS

Like most of the early work on the innervation of the salivary glands, this investigation has been carried out on dogs. In the present case this species was chosen since reflex stimulation of its salivary glands is fairly easy and because it seemed likely that important secretory pathways outside the auriculo-temporal nerve existed (Babkin, 1950). There are, however, some indications of such nerves in other species too. Thus Nordenfelt (1963) found that, in the rabbit parotid, 3 percent of the normal choline acetyltransferase remained after division of the auriculo-temporal nerve and, in cat parotid, 10 percent. This suggests that there are more cholinergic secretory nerves outside the auriculo-temporal nerve in the dog than in the other two species. In the cat, parotid secretory nerves have been traced on the internal maxillary artery (Ekström & Emmelin, personal communication). The parotid secretory innervation in man has attracted considerable interest, because division of it has been used in the treatment of clinical conditions such as Frey's syndrome, "the syndrome of crocodile tears" and nonsuppurative recurrent parotitis (see Golding-Wood, 1962). In 1927 Dandy noticed that salivary secretion was unaffected by intracranial section of the glossopharyngeal nerve for the relief of neuralgia. Reichert and Poth (1933), on the other hand, found that parotid secretion was reduced both after intracranial section of the glossopharyngeal nerve and of the chorda tympani in the middle ear and concluded that these two nerves carry secretory fibres for the gland. Diamant and Wiberg (1965) have presented evidence which supports such an opinion. The more peripheral course of the secretory fibres in man is unknown, but it is assumed to be in the auriculo-temporal

nerve, the neurones of which are said to originate in the otic ganglion. Chorda tympani fibres may reach this ganglion (Reichert & Poth, 1933) via the so-called Spalteholz's anastomosis, just distal to the Glaserian fissure. The parotid secretory fibres branching off from the human facial nerve in the chorda tympani might correspond to those which continue in the peripheral part of the facial nerve in the dog.

Contrary to many other salivary glands, the dog's parotid does not seem to have a sympathetic secretory innervation. This was noticed early (Bernard, 1879 ; Navrocki, 1868; Heidenhain, 1878) and later confirmed (Emmelin, Ohlin & Thulin, 1969), but some investigators report secretion from the gland in response to sympathetic stimulation (Moussu, 1890; Shimamoto & Inoue, 1958) and adrenergic drugs (Shimamoto & Inoue, 1958). The present findings with complete abolishment of reflexly induced parotid secretion after atropine supports the opinion that the gland does not receive any sympathetic secretory nerves. It might then seem curious that there are more catecholamine fluorescent nerves in the dog's than in the cat's parotid (Garrett & Holmberg, 1972) which, at least according to some, secretes in response to sympathetic stimulation. Sympathetic nerves, however, innervate myoepithelial cells (see Emmelin, Garrett & Ohlin, 1969) and vessels (Claude Bernard, 1879) in the dog's parotid, and furthermore, modify the composition of the salivary secretion mediated via parasympathetic nerves. Thus Heidenhain (1878) found that after sympathetic stimulation there were changes in the microscopical appearance of the glandular cells, suggesting secretory activity. He also noticed that the content of organic constituents in the parotid saliva increased when sympathetic stimulation was superimposed upon secretion provoked by electrical excitation of the tympanic nerve.

It turned out, that the key to the problem of the secretory innervation of the dog's parotid was to be sought in the secretory fibres on the internal maxillary artery described by Claude Bernard. In the work on this problem, a preparation has been worked out where practically all the pre- and post-ganglionic secretory nerves can be stimulated and divided. It was, however, not always possible to remove all the secretory nerves. It seems that no salivary gland of any animal species has hitherto been convincingly totally denervated. As for the submandibular gland of the dog, Seo (1934) divided the chorda tympani, glossopharyngeal and splanchnic nerves, the nerves to the liver and thyroid gland, decapsulated the submandibular, "cleaned" its vessels and removed the superior cervical ganglion but still noticed a slight secretion from the submandibular gland in response to feeding. The problem of the course of the secretory nerves is a difficult one; its history contains names such as Claude Bernard, Eckhard and Heidenhain. Yet, after summing it up in 1898, Langley remarks: "If the secretory fibres of the parotid really arise from the ninth nerve, the majority of the early observations form a singular record of inadequate experiments and hasty deductions". Half a century later Babkin (1950) wrote: "The whole problem of the innervation of the salivary glands requires re-investigation". The present work was prompted by this statement and by the realization that a detailed knowledge of the innervation is essential when these glands are used as model organs for studies of secretory and neuro-effector functions.

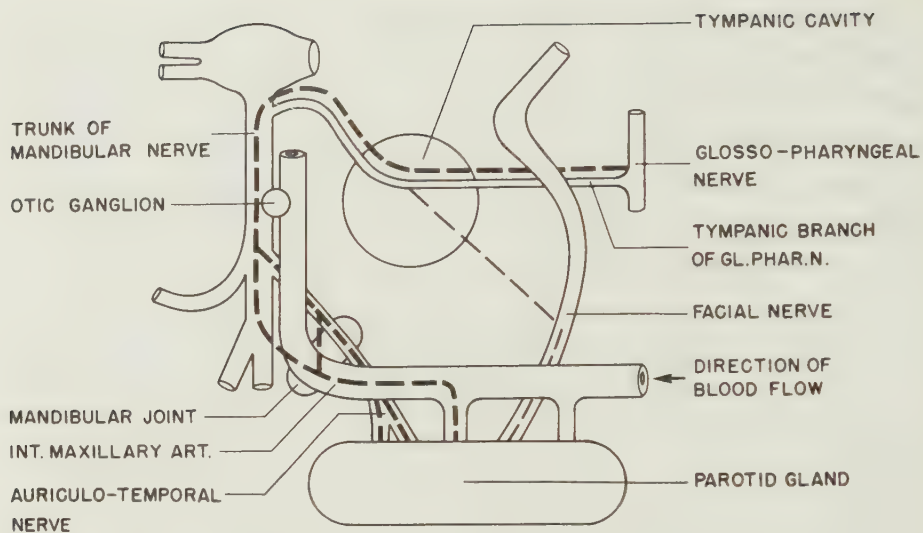


Fig. 1. Diagram showing the course of the secretory nerves to the dog's parotid.

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